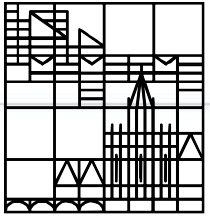


Mapping the Human Toxome by Systems Toxicology Using ED as Proof of Concept

Thomas Hartung

Doerenkamp-Zbinden Professor and Chair for Evidence-based Toxicology, EHS
Director, Center for Alternatives to Animal Testing (CAAT)
Joint appointment: Molecular Microbiology and Immunology
Bloomberg School of Public Health, Johns Hopkins University, Baltimore, US

Professor of Pharmacology and Toxicology, University of Konstanz, Germany

About 1/3 funding each from industry, philanthropy and research funding

ALLERGAN

RIFM

IFRA INTERNATIONAL FRAGRANCE ASSOCIATION

BDF Beiersdorf

Johnson & Johnson

Agilent Technologies

NOVARTIS

Abbott A Promise for Life

Pfizer Working together for a healthier world™

ExxonMobil

Boehringer Ingelheim

L'ORÉAL

Roche

P&G

Unilever

BASF The Chemical Company

The Bernice Barbour Foundation

DOERENKAMP-ZBINDEN Foundation

THE ESTHER A. & JOSEPH KLINGENSTEIN FUND, INC.

…and individuals

EUROPEAN COMMISSION

FDA

NATIONAL INSTITUTES OF HEALTH

DEFENSE THREAT REDUCTION AGENCY UNITED STATES OF AMERICA

JOHNS HOPKINS BLOOMBERG SCHOOL of PUBLIC HEALTH

The 10.000 foot perspective

100,000 in commerce <3% tested for endocrine or reproductive tox.

Human endocrine health effects including cancer?

Endocrine disruption

ECVAM

- **Task force**
- **QSAR workshop**
- **Validation**
- **ReProTect**

CAAT

- **White paper**
- **NIH consortium**
- **Conference Rome 17 Dec 2012**

transatlantic think tank for toxicology

Ambassadors

Bas Blaauboer
Alan Goldberg
Thomas Hartung
Marcel Leist

The pipeline

13 articles published (5 responses in Nature / Science)

6 articles in preparation

7 workshop reports published
6 reports pending

3 workshops planned

7'2010: 21st Century Validation for 21st Century Tools

Courage for Simplification and Imperfection in the 21st Century Assessment of "Endocrine Disruption"

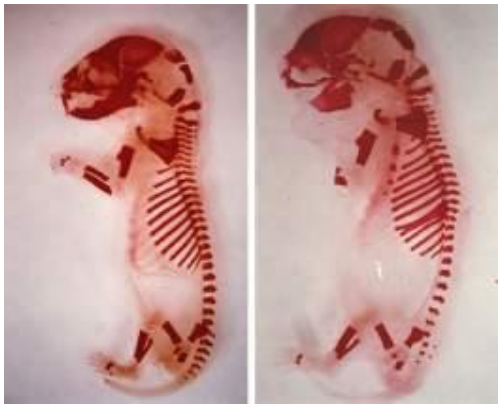
Daniel R. Dietrich
Human and Environmental Toxicology and CAAT-Europe, University of Konstanz, Germany

All current toxicological information to date strongly suggest that the routine rodent models are inadequate for detection of EAS and assumed endocrine effects in humans

Hershberger & uterotrophic

- **Not sensitive enough**
- **Unsuitable validation design**
- **Abrogation of peer-review**

Animal test Reprotox



8 months

$0.6 million/chemical

3,200 animals/chemical

64% positive

why?

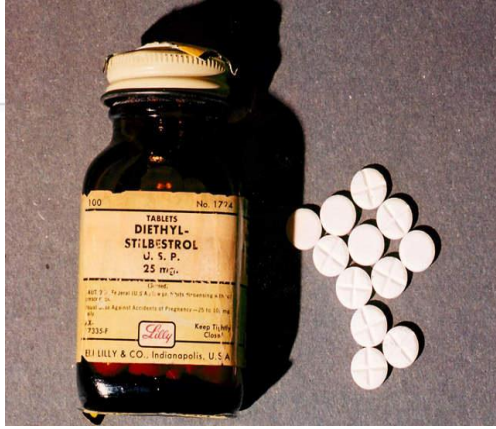
Estimate human 2-3% positive

Problem inter-species differences

mice, rat, rabbit, guinea pig predict each other

with 60% correlation

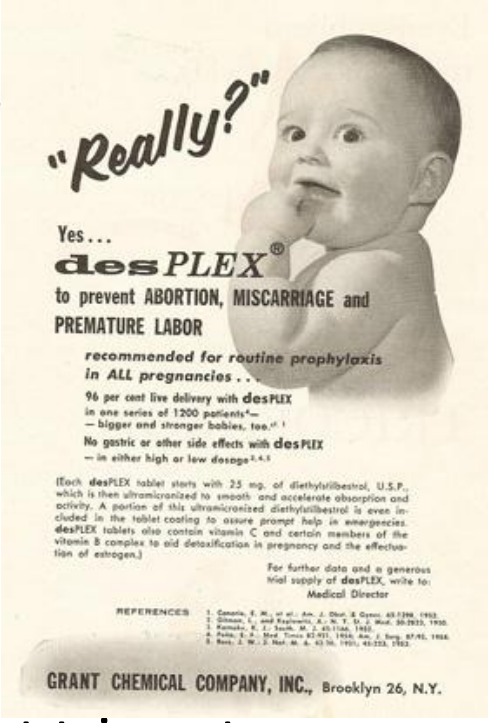
Diethylstilbestrol (DES)



- Stilbene, synthetized in 1938, with structural similarities to E2
- Prescribed from the 1940‘s to beginning of 1970‘s to pregnant women to prevent spontaneous abortion and preterm delivery
- Estimates: 5-10 million fetuses exposed in the USA and Europe combined
- Teratological effects: Urogenital abnormalities in male and female offspring incl. infertility, clear-cell cervico-vaginal cancer in female offspring

Slides courtesy of D. Dietrich, Konstanz

Diethylstilbestrol (DES) A more detailed analysis



- > 80% of female offspring with urogenital abnormalities when exposed to cumulative dose ≥ 12000 mg within 1st 9w of gestation
- Urogenital abnormalities and clear-cell cervico-vaginal cancer in female offspring when exposed to cumulative dose ≥ 5000 mg within 1st trimester.
- However, median cumulative of 2530 mg over whole gestational period NOT associated with urogenital abnormalities in female offspring
- DES binds to human sex hormone binding globulin (shbg) with >250 lower affinity than E2
- DES has a relative binding affinity to ERα of 17% of E2 – E2 displacing capacity at high endogenous concentrations

Diethylstilbestrol (DES) A resource untouched?

- The US Diethylstilbestrol Adenosis (DESAD) Project reviewed approx. 5000 cases (of 5-10 Mio exposed?)
- Of the cases reviewed, the incidence for urogenital abnormalities ranges between 1,57 and 65% (control 0,79%)!
- The reported risk of clear-cell cervico-vaginal cancer ranges between 1/1000 and 1/10‘000 (Giusti et al 1995)
- Current data strongly suggest that even high doses of DES (short exposure period) or moderate doses (prolonged exposure period) had NO ADVERSE EFFECTS!
- What could a detailed investigation offer?
  - Patient data re consumption and possible clinical data re levels: pharmacokinetic parameters
  - NOAEL calculations for specified time-periods of exposure

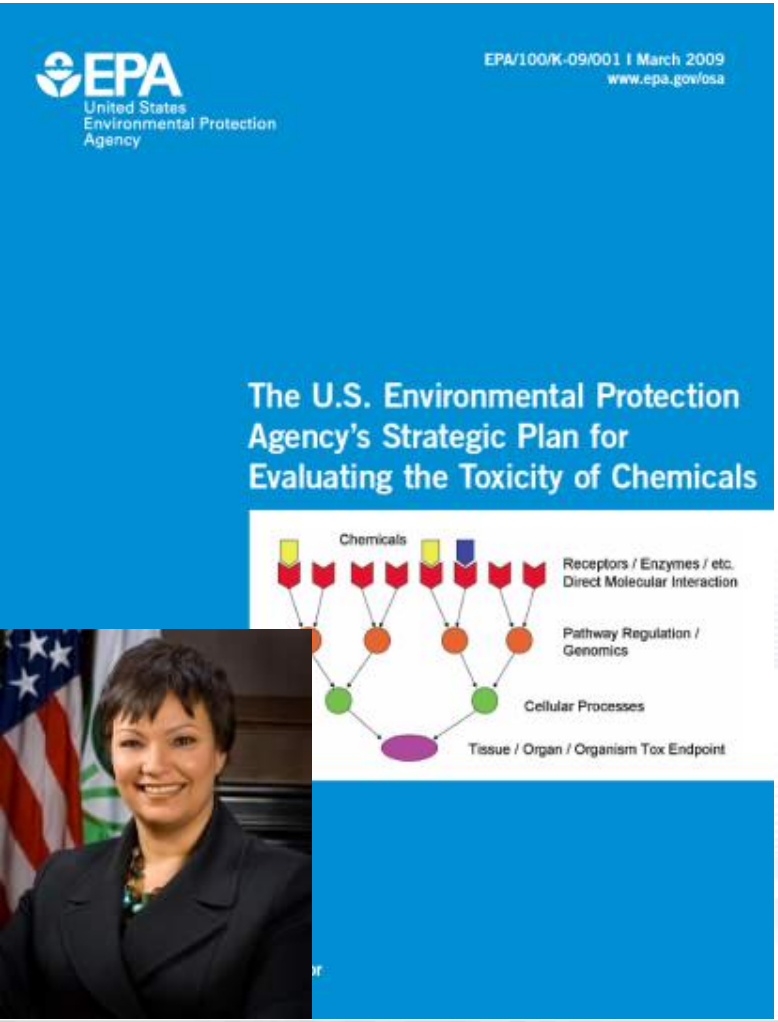
Lessons learnt from „causal“ EAS exposure and effect examples in humans

- *The example of DES thus elegantly demonstrates that even for the most potent EAS in humans (with regard to endocrine disruption) known to date, it is primarily a “high dose, specific activity, and prolonged time during a critical period principle” that governs the manifestation of “endocrine disruptive effects” in humans.*
- *The current data IN HUMANS for steroids employed as contraceptives, SERMs, glitazones (4mg/d) or ketoconazole (600-1000mg/d) DO NOT provide any evidence that even in short-term overdose situations during gestation, endocrine disruptive effects occur in the offspring*
- *All current toxicological information to date strongly suggest that the routine rodent models are inadequate for detection of EAS and assumed endocrine effects in humans*

Serious doubt remains as to a major impact of more than a few chemicals as ED

But society wants problem addressed

NAS vision report Tox-21c



"With an advanced field of regulatory science, new tools, including functional genomics, proteomics, metabolomics, high-throughput screening, and systems biology, we can replace current toxicology assays with tests that incorporate the mechanistic underpinnings of disease and of underlying toxic side effects." **M.A. Hamburg, FDA 2011**

"We propose a shift from primarily in vivo animal studies to in vitro assays, in vivo assays with lower organisms, and computational modeling for toxicity assessments"
F. Collins, NIH, 2008

Initiatives implementing Tox-21c

Organization	Approach	Purpose	Outcome
US EPA & Tox21 (ToxCast Program)	High-throughput testing	Chemical prioritization (initially)	"Biological signatures"
Hamner Institute	Case studies	"Just do it"	Proof-of-principle
NIH project (CAAT-US)	Pathway mapping	Pathway ID & annotation	Human Toxome

Tox-20c

EBT

Omics, high-content, HTS Bio-informatics & -engineering

Tox-21c

Pathways of Tox (PoT)
Human Toxome

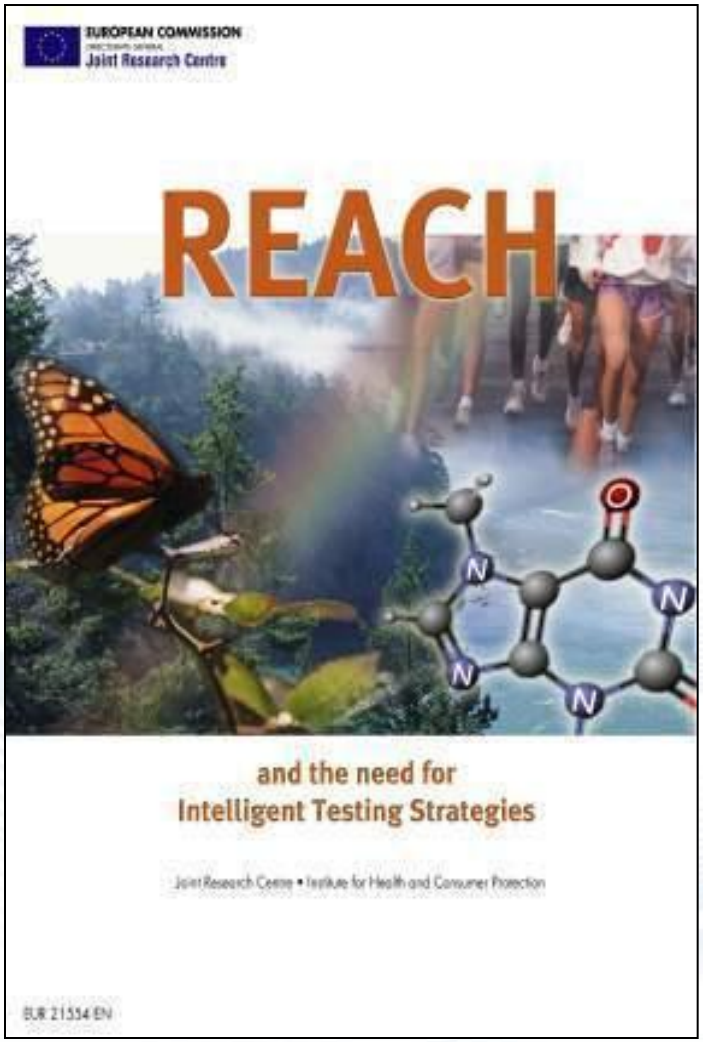
Integrated Testing Strategies
ITS

Organo-typic cultures
Human-on-Chip

Scientific roadmap for the future of animal-free systemic toxicity testing

US Stakeholder Forum 30-31 May 2013
Hosted by FDA CFSAN
Looking for further interested partners!!!

Integrated Testing Strategies



- **Key contribution to REACH implementation process**
- **Use of different informations, not stand-alone replacement**

Just became available (AltWeb or ALTEX website)

Food for Thought ...
Integrated Testing Strategies for Safety Assessments

Thomas Hartung [1,2], *Tom Luechtefeld* [1], *Alexandra Maertens* [1], *and Andre Kleensang* [1]

[1]Johns Hopkins University, Bloomberg School of Public Health, CAAT, Baltimore, USA; [2]University of Konstanz, CAAT-Europe, Germany

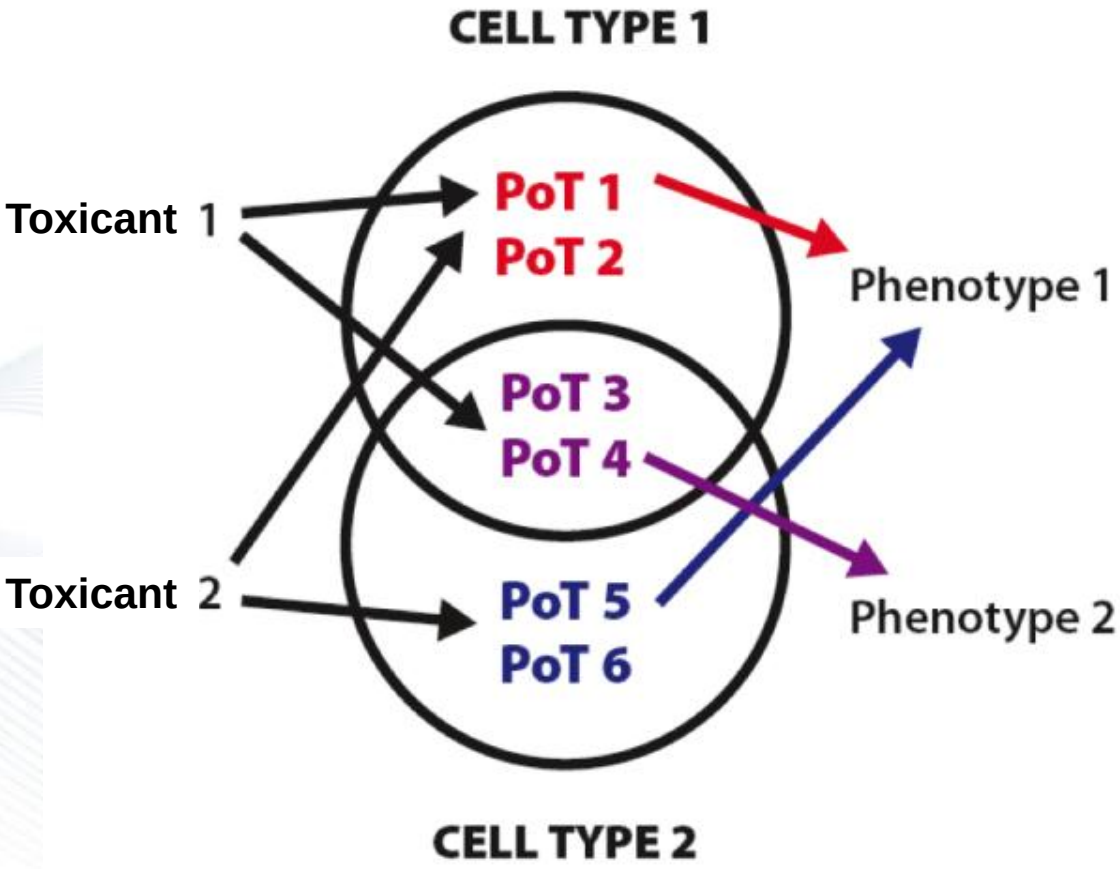
WoE, EBT, ITS…. Similar problems, but not the same

all: quality and data integration problem

EBT/WoE retrospective -- ITS prospective

WoE pragmatic -- EBT / ITS formalized

The concept of (finite number of) pathways of toxicity



Annotation to:

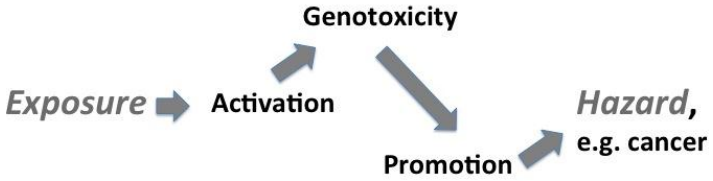
- **Hazard**
- **Toxin (class)**
- **Cell type**
- **Species**

Comprehensive list (Human Toxome) → **Negatives**

A) Phenomenological Toxicology

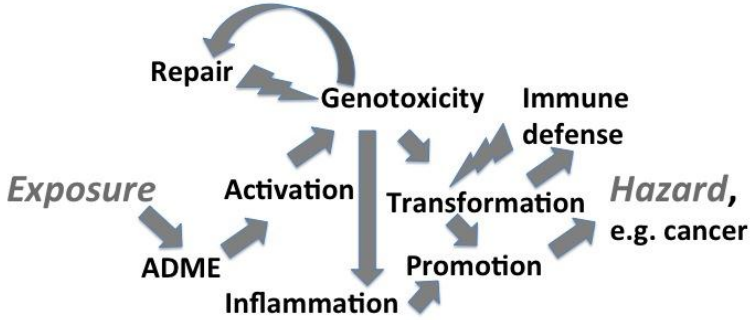
Causality supported by correlation and e.g. Bradford-Hill criteria

B) Mode of Action Toxicology (e.g. Adverse Outcome Pathways)



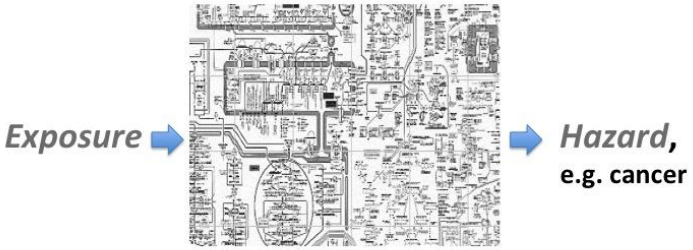
Causality supported by experimental intervention, e.g. Koch-Dale approaches

C) Mechanistic Toxicology (toxicity pathways)

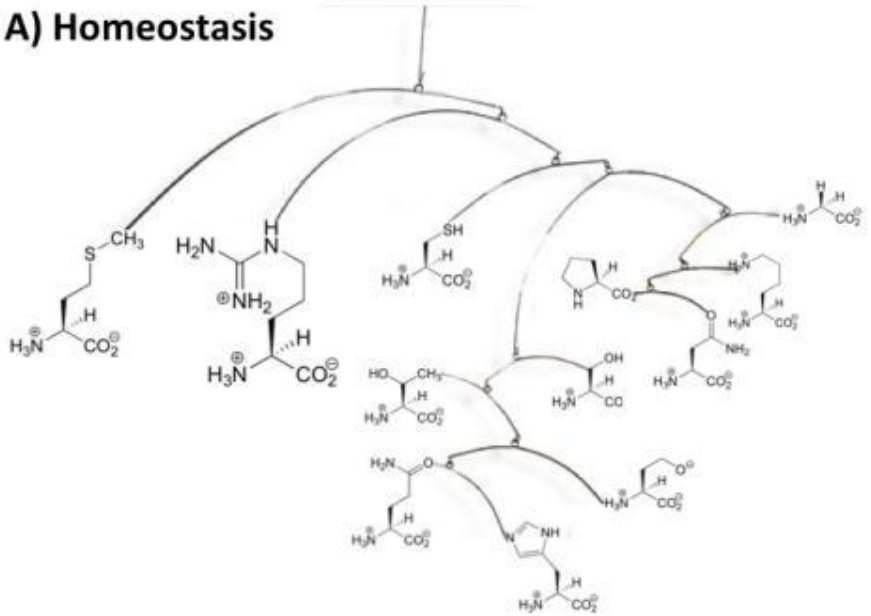


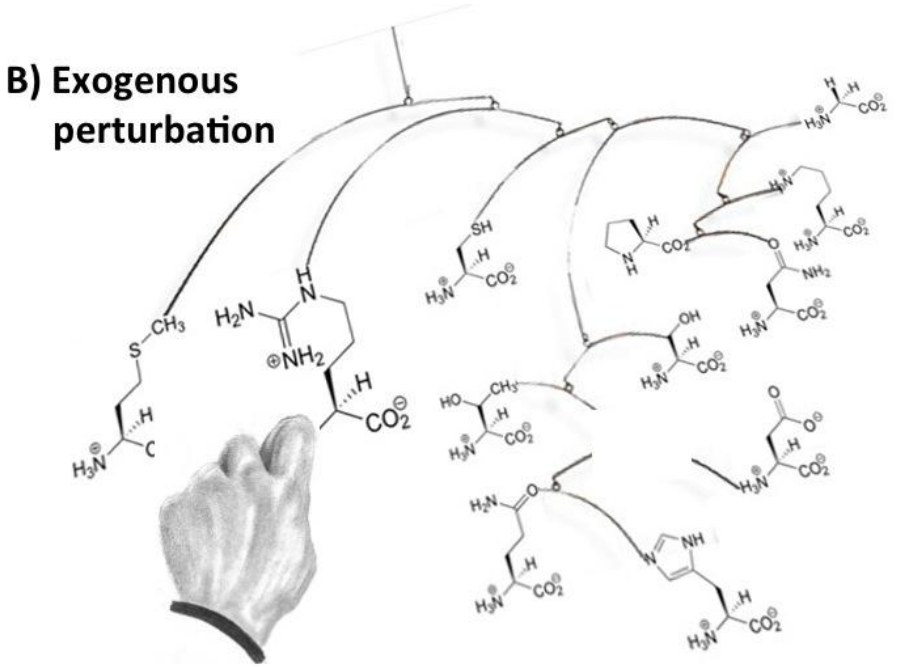
Causality supported by simulation and other bioinformatic tools

D) Systems Toxicology (resolution on PoT and fluxes)



Systems Toxicology approaches





Use for PoT identification:

- **Homeostasis under stress, i.e. signatures of tox**
- **Critical cell infrastructures**
- **Network knowledge**
- **Reference models**
- **Reference toxicants**

NIH Transformative Research Grant: *Mapping the Human Toxome by Systems Toxicology*

Consortium:

- **Johns Hopkins** (Hartung / Yager)
- **Brown** (Boekelheide)
- **The Hamner** (Andersen)
- **Georgetown** (Fornace)
- **Agilent** (Rosenberg)
- **EPA ToxCast** (Kavlock, Dix)

Mapping PoT from metabolomics and transcriptomics

In vitro systems

- Toxicological endpoints
- Model Systems Characterization
- In vitro treatments

omics data generation

- HC omics data Metabolomics &Transcriptomics

Software tools

- Software for Statistics and Pathways
- Visualization Tools

Pathways of Toxicity

- Signature of Toxicity Analysis
- PoT Concept Development

Human Toxome Database

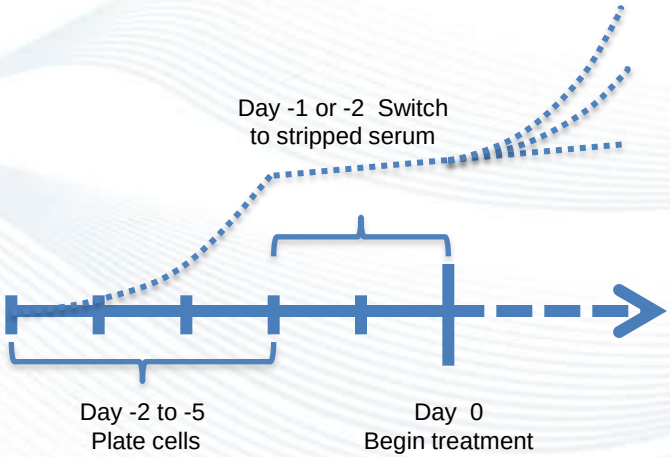
- Pathway Validation
- IV-IV Extrapolation
- Toxcast, other data

(Pre-)Validated work

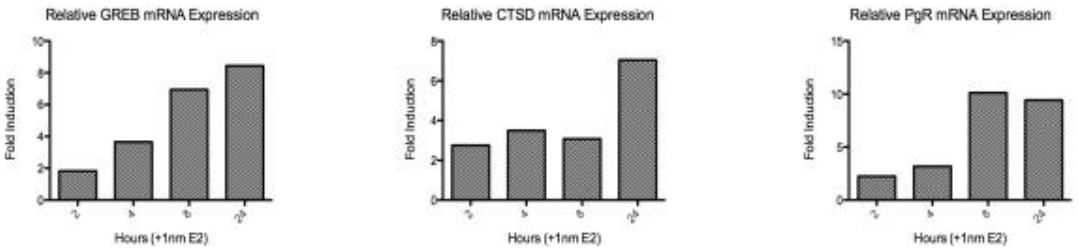
- **Robust protocols, good cell models**
- **Regulatory acceptance available or in progress**
- **Available reference substances**
- **Thresholds of adversity defined**

In vitro systems

- **MCF-7 cells (currently undergoing validation by ICCVAM) &**
- **Initial set of endocrine disrupting chemicals selected from a priority list of 53 reference compounds identified by ICCVAM**

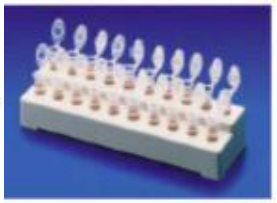


Cell culture SOP



Gene Expression – Timecourse
1nM estradiol – 2, 4, 8, and 24 hours

MCF-7 cell culture and E2 exposure



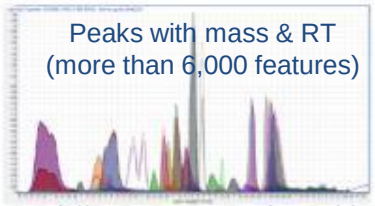
Sample prep and metabolite extraction

Data acquisition (Q-Tof)

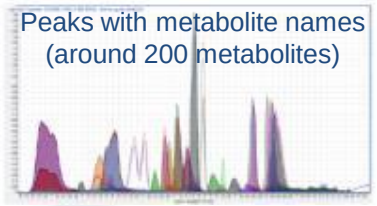
omics data generation

Untargeted

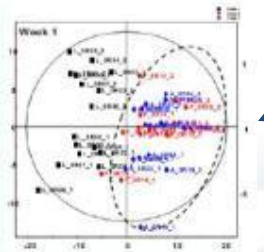
Targeted



Peak/feature extraction with NaÏve data mining algorithm

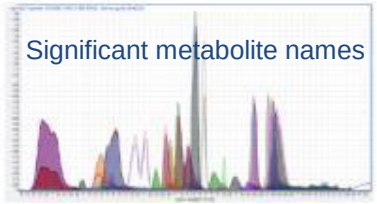


Metabolite peak extraction with database searching

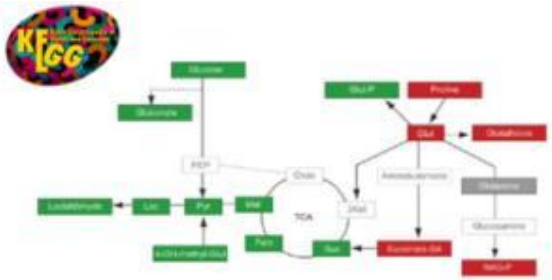


Bio-statistical analysis (e.g. ANOVA, PCA and clustering analysis)

Workflow for Metabolomics



Metabolites Identification (only for untargeted) and confirmation (for both approaches)



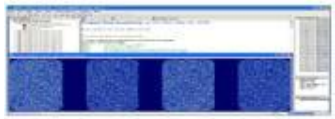
Data interpretation (e.g. Pathway analysis)

Software tools

LC/MS
GC/MS

MassHunter Qual/Quant
ChemStation AMDIS

Microarrays



Feature Extraction

NGS

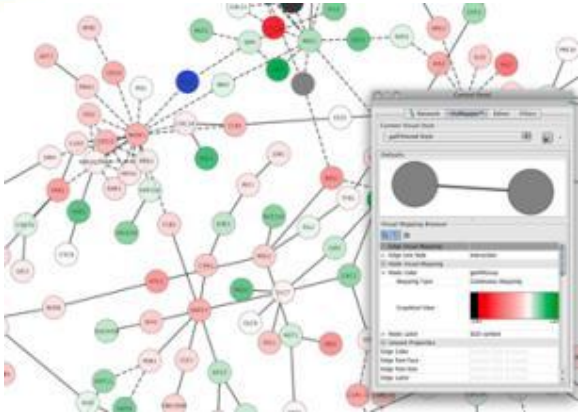
Alignment to Reference Genome

GeneSpring Platform

Biological
Pathways

Agilent Integrated Biology Workflows

Software tools

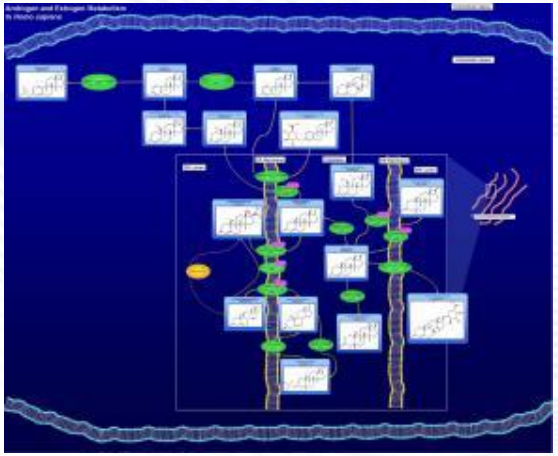


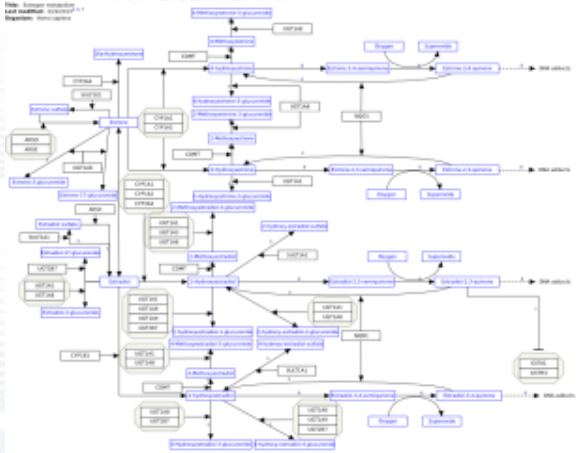
Cytoscape

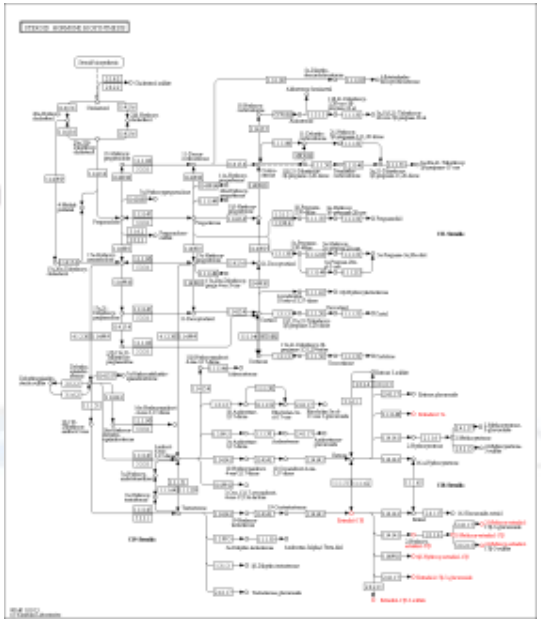
SMPDB
Small Molecule Pathway Database

WIKIPATHWAYS
Pathways for the People

KEGG
Kyoto Encyclopedia of Genes and Genomes

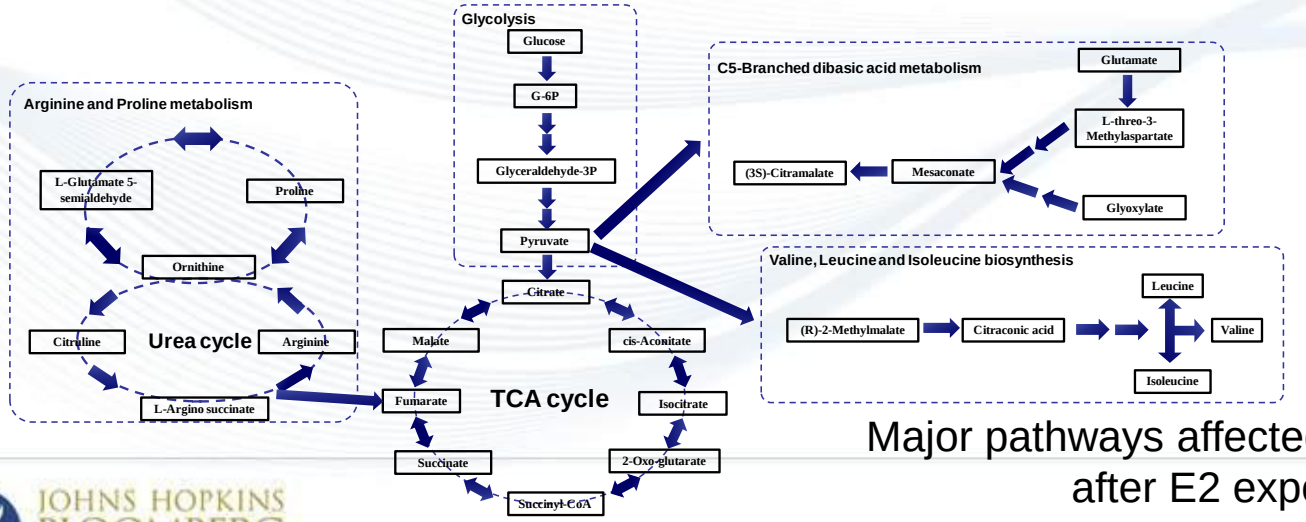






Significant Metabolites	Related Pathways
Malate	Citrate cycle (TCA cycle)
Fumarate	Citrate cycle (TCA cycle); Amino acid metabolisms
Aconitate	Citrate cycle (TCA cycle); C5-Branched dibasic acid metabolism
Pyruvate	Citrate cycle (TCA cycle); Glycolysis / Gluconeogenesis; Amino acid metabolisms
D-glyceraldehdye-3-phosphate	Glycolysis / Gluconeogenesis
Lactate	Glycolysis / Gluconeogenesis
Citraconic acid	Valine, leucine and isoleucine biosynthesis; C5-Branched dibasic acid metabolism
L-threo-3-Methylaspartate	C5-Branched dibasic acid metabolism
Glyoxylate	C5-Branched dibasic acid metabolism
(R)-2-Methylmalate	Valine, leucine and isoleucine biosynthesis; C5-Branched dibasic acid metabolism
Arginine	Arginine & Proline Metabolism
Valine	Valine, leucine and isoleucine biosynthesis
L-Glutamate 5-semialdehyde	Arginine & Proline Metabolism
Ornithine	Arginine & Proline Metabolism
Leucine/Isoleucine	Valine, leucine and isoleucine biosynthesis

Pathways of Toxicity



Major pathways affected in MCF-7 cells after E2 exposure

PROPOSAL FOR A TEMPLATE, AND GUIDANCE ON DEVELOPING AND ASSESSING THE COMPLETENESS OF ADVERSE OUTCOME PATHWAYS

2012

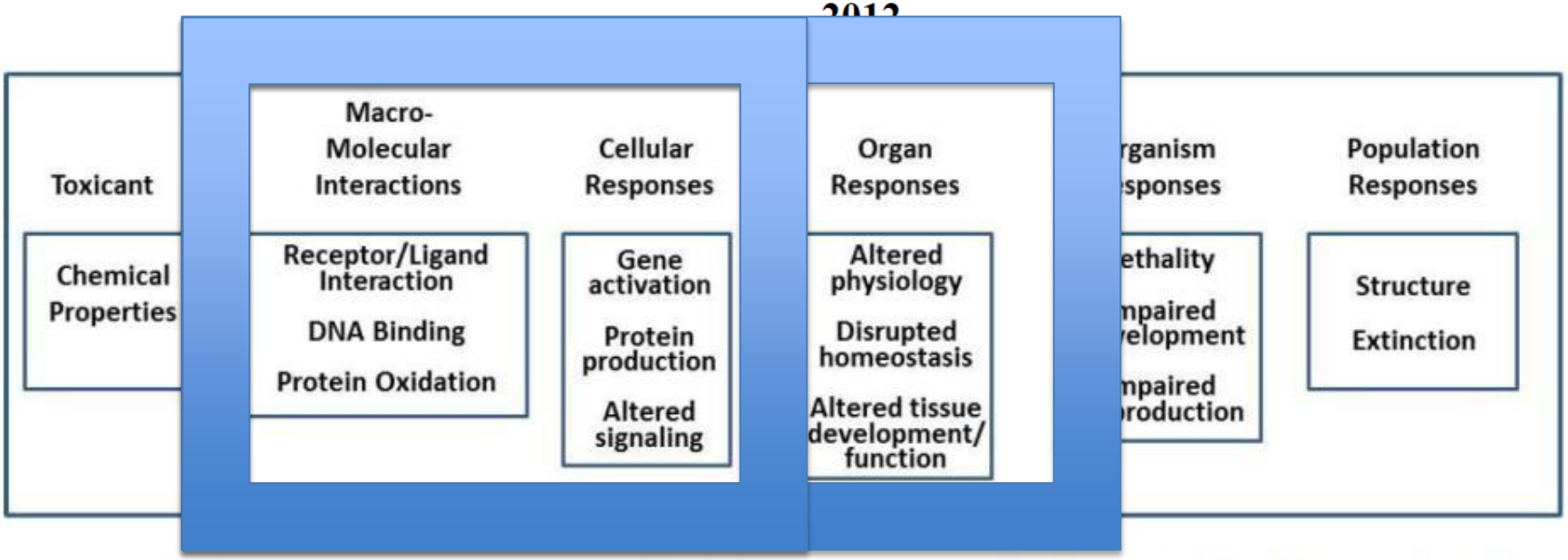
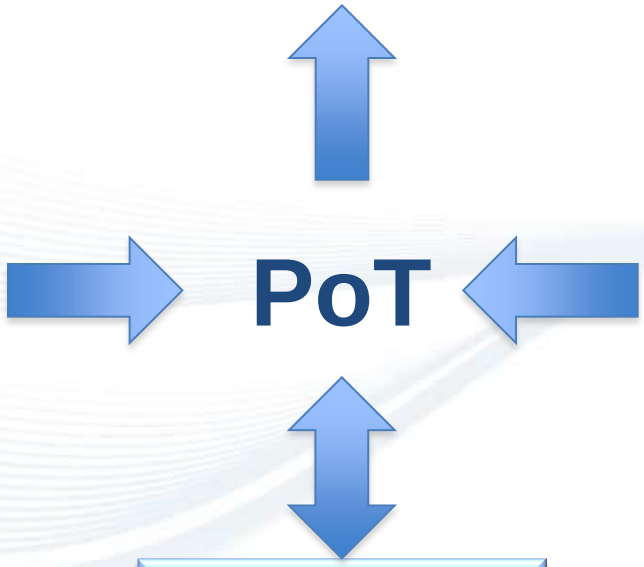


Figure1. A schematic representation of the Adverse Outcome Pathway (AOP) illustrated with reference to a number of pathways.

PoT

Workshop on the Concept and Tools for Pathways of Toxicity
October 10 -12, 2012, Baltimore, MD

Human Toxome database



Content side:
- Mol.biol.
- Biochem.
- Omics SoT
- Tox Mechan.

User side:
- Regulation
- Probabilistic RA
- Systems Toxicology
- Virtual patient

Existing databases

PoToMaC - The Pathways of Toxicty Mapping Center

European branch?

Transformative Research Grant:

Mapping the Human Toxome by Systems Toxicology

HUMAN TOXICOLOGY PROJECT CONSORTIUM

7 companies, 3 stakeholders

2 Mar 2012

"Driven both by legislative mandate and scientific need, a new suite of in vitro and cell culture-based animal-free methods are gaining a foothold in toxicology labs."

LIFE SCIENCE TECHNOLOGIES

Produced by the *Science*/AAAS Custom Publishing Office

Toxicology

Animal-Free Toxicology

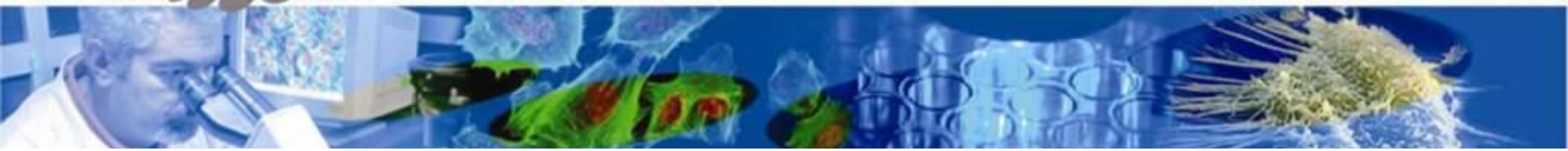
Sometimes, in Vitro is Better

The next time you use shampoo, air freshener, or moisturizing cream, consider this: How do you know it's safe? In all likelihood, whatever toxicologic screening its component ingredients were subjected to involved laboratory animals, the method of choice for decades and the industry's reigning "gold standard." Yet as Bob Dylan once put it, the times, they are a-changing. Animal-based testing is expensive and time-consuming, morally and ethically troubling, and most significantly, often a poor predictor of human toxicity. Animals aren't going anywhere just yet. But their numbers are dropping. Driven both by legislative mandate and scientific need, a new suite of in vitro and cell culture-based animal-free methods are gaining a foothold in toxicology labs. **By Jeffrey M. Perkel**

One key player in the modernization of toxicology screening is automation.

EBT

1st International Forum towards
Evidence-Based Toxicology (EBT)
October 15-18, 2007, Como, Italy



Evidence-based Toxicology

"Evidence-based medicine goes toxicology!"

Hoffmann and Hartung "Toward an evidence-based toxicology", Human Exp. Tox., 2006

Mar 2011: US EBTC
Oct 2011: Secretariat at CAAT
Jan 2012: First conference hosted by EPA

Kick-off meeting of the Evidence-Based Toxicology Collaboration (EBTC) Europe

ebtc
Evidence-based Toxicology Collaboration

In conjunction with Eurotox Congress 2012 (Stockholm, Sweden)

June 17, 2012
15:30h - 17:30h

Radisson Blu Royal Viking Hotel • Vasagatan 1, Stockholm, Sweden
Complimentary Registration: http://www.ebtox.com

Johns Hopkins University Center for Alternatives to Animal Testing

EBT Collaboration Steering Committees

EBTC

www.ebtox.com

Wiktionary LEO Pubget Google Scholar Welch Welch Library K-finder Slideshare Prezi Google Maps News Wikipedia Wiktionary KODAK PULSE Thomas Evernote tom Polular Popular

Detection and sequencing of microRNA using MALDI time-of-flight mass spectr... | libres.uncg.edu/ir/uncg/f/Yang_uncg_0154M_10557.pdf | EBTC

RSS Twitter Facebook

ebtc
Evidence-based Toxicology Collaboration

About Us Contact Us Meetings & Symposia Steering Committee

What is Evidence-based Toxicology?

The Evidence-Based Toxicology (EBT) Collaboration has recently taken up the challenge of translating evidence-based approaches from medicine to toxicology. The Collaboration has closely coordinated steering committees in the US and Europe with members drawn from government agencies, academia, and industry. More. . . .

LATEST NEWS

US EBTC Receives Informal Tutorial on Systematic Reviews
The US EBTC Steering Committee held an informal tutorial on systematic reviews (SRs) on July 23, 2012 at Johns Hopkins S...

Kick-off meeting of the Evidence-Based Toxicology Collaboration (EBTC) Europe
In conjunction with Eurotox Congress 2012 (Stockholm, Sweden) June 17, 2012 | 15:30h - 17:30h Radisson Blu Royal Vi...

Definition of Validation

ALTEX 27 (2010) 253-263

Evidence-Based Toxicology – the Toolbox of Validation for the 21st Century?

Thomas Hartung
Johns Hopkins University, Bloomberg School of Public Health, Dept. Environmental Health Sciences, Center for Alternatives to Animal Testing (CAAT), Doerenkamp-Zbinden Chair for Evidence-based Toxicology, Baltimore, MD, USA, and Professor of Pharmacology and Toxicology, University of Konstanz, Germany

Knowledge:
- PoT
- MoA

Just became available (AltWeb or ALTEX website)

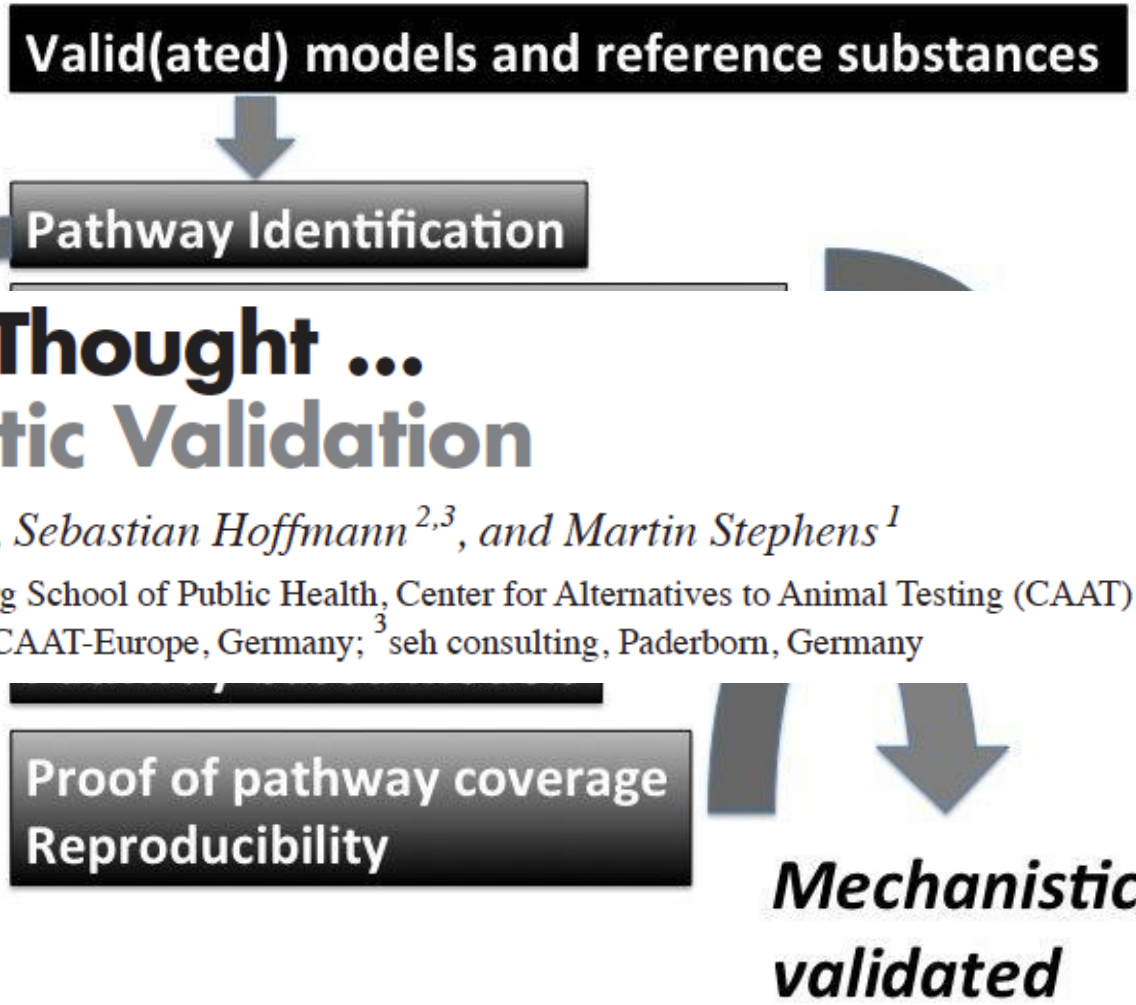
Workshop Report

Evidence-based Toxicology for the 21st Century: Opportunities and Challenges*

Martin L. Stephens [1], Melvin Andersen [2], Richard A. Becker [3], Kellyn Betts [4], Kim Boekelheide [5], Ed Carney [6], Robert Chapin [7], Dennis Devlin [8], Suzanne Fitzpatrick [9], John R. Fowle III [10], Patricia Harlow [11], Thomas Hartung [1], Sebastian Hoffmann [12], Michael Holsapple [13], Abigail Jacobs [11], Richard Judson [14], Olga Naidenko [15], Tim Pastoor [16], Grace Patlewicz [17], Andrew Rowan [18], Roberta Scherer [1], Rashid Shaikh [19], Ted Simon [20], Douglas Wolf [14], and Joanne Zurlo [1]

Perspectives on Validation of High-Throughput Assays Supporting 21st Century Toxicity Testing

Richard Judson [1], Robert Kavlock [1], Matthew Martin [1], David Reif [1], Keith Houck [1], Thomas Knudsen [1], Ann Richard [1], Raymond R. Tice [2], Maurice Whelan [3], Menghang Xia [4], Ruili Huang [4], Christopher Austin [4], George Daston [5], Thomas Hartung [6], John R. Fowle III [7], William Wooge [8], Weida Tong [9], and David Dix [1]



Food for Thought ...

Mechanistic Validation

New ALTEX

Thomas Hartung [1,2], *Sebastian Hoffmann* [2,3], *and Martin Stephens* [1]

[1]Johns Hopkins Bloomberg School of Public Health, Center for Alternatives to Animal Testing (CAAT), Baltimore, MD, USA; [2]University of Konstanz, CAAT-Europe, Germany; [3]seh consulting, Paderborn, Germany

Johns Hopkins is the right environment for EBTC secretariat

The difficulty lies, not in the new ideas, but in escaping from the old ones.

John Maynard Keynes

(1883 - 1946)